Protein Targeting Study Question Answers

Question. 1. Describe the targeting signals for entry into the RER, for incorporation into the RER membrane, for recycling from the Golgi to the RER, for targeting to lysosomes, mitochondria, the nucleus, and the peroxisome.

a) Entry into the RER: the signal peptide which consists of a 7 to 13 aa hydrophobic sequence flanked by hydrophilic residues, in particular a basic residue or two on the N-terminal side of the signal peptide. The signal peptide is followed by a small amino acid like alanine, glycine or serine which immediately precedes the cleavage site for signal peptidase.

b) RER membrane incorporation--proteins that are destined to be integral membrane proteins contain a signal peptide and an additional sequence called a stop transfer sequence which consists of a hydrophobic α-helix.

c) Recycling signal is KDEL (lysine-aspartate-glutamate-leucine) found in the cytoplasmically exposed C-terminus of RER resident proteins.

d) Targeting to lysosomes from the secretory pathway requires a mannose 6-phosphate moiety on the protein and this is commonly found on lysosomal hydrolases. The signals for targeting to the lysosome via endocytosis (whether receptor-mediated or not) and by autophagy were not discussed.

e) The targeting signal for mitochondrial import is less well defined than most. It usually consists of an N-terminal stretch of amino acids rich in positively charged amino acids, serines and threonines.

f) Targeting to the nucleus requires a string of about five consecutive basic residues found somewhere in the cytosolic protein.

g) The peroxisome targeting sequence is an SKF (serine-lysine-phenylalanine) motif found in the C-terminus of the cytosolic protein.

Question 2. What is the major difference in the amino acid sequence of a protein imported into the lumen of the RER from one which is inserted into the RER membrane?

For a protein to be incorporated into the RER membrane it usually contains a signal peptide and **an additional sequence called a stop transfer sequence which consists of a hydrophobic α-helix**.

Question 3. List at least three families of GTPases involved in protein trafficking / targeting? In which pathway do they each function?

a) ARF GTPase: functions in vesicle budding from RER and Golgi membrane. The example given in lecture was for vesicle budding from the RER.
b) Rab GTPase: functions in vesicle docking and fusion perhaps by readying SNAREs to pair. The example given in class was for a vesicle docking and fusing with the *cis* Golgi.

NOTE: For the ARF and Rab GTPases, their cycle of GTP-binding and hydrolysis is coupled to vesicle formation and fusion, respectively. This ensures that transport proceeds from the prior compartment to the subsequent compartment. Their biochemical activities ensure that transport occurs in a vectorial manner.

c) Dynamin: Involved in severing endocytic vesicles from the plasma membrane. Involved in endocytic vesicle production.

d) Ran GTPase required for nuclear import.

Question 4. What are the two main degradative pathways for proteins within the cell?

Lysosomal pathway and the ubiquitin / 26S proteosome pathway

Question 5. Describe at least two processes which involve endocytosis.

a) Nutrient uptake. The example given in class is for iron uptake by receptor mediated endocytosis of transferrin by the transferrin receptor. Pinocytosis, which is not receptor mediated is also used for nutrient uptake.

b) Endocytosis is also a pathway that some RNA viruses use to gain entry to the cell.

c) Phagocytosis, which is sort of a macro form of endocytosis, is also used by some cells (macrophages and polymorphonuclear leukocytes) to engulf and kill foreign bacterial and to degrade debris produced during wound healing.

Question 6. List the clinical / militarily relevant examples given in this lecture.

a) Protein folding disorders: alpha1AT defect and Δ508 allele of cystic fibrosis. The proteins misfold in the RER and are prevented from getting to their final sites of action. Instead, they are exported from the RER and degraded in the cytoplasm.

b) I-cell disease. Results from a defect in the phosphotransferase found in the medial Golgi. Without this modification, the lysosomal hydrolases are secreted from the cell instead of being transported to the lysosome.

c) Biological toxins, tetanus and botulinum toxins, block neurotransmission by destroying the neuronal specific SNARE proteins which prevents neurotransmission.

d) Nutrient uptake. Uptake of iron by receptor-mediated endocytosis

e) Virus entry through the endocytic pathway. The example given in class was for the *Semliki forest* virus.